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The pathogenesis of osteonecrosis in sickle cell anemia (SCA) remains unknown. Blood hyper-viscosity has been suggested as a factor involved in the genesis of osteonecrosis[1], but not studied until now. We hypothesized that abnormal hemorheology could play a role in this complication. Hematological and hemorheological parameters were assessed in SCA patients with osteonecrosis (OST+, n=30) or without (OST-, n=67). Osteonecrosis was diagnosed as previously described[2]. The study was conducted according to the Declaration of Helsinki guidelines, and was approved by the Regional Ethics Committee. The results are reported in the table. OST+ patients were older than OST- ($p < 0.05$) and more had a history of vaso-occlusive crises (VOC) within the previous year ($p < 0.05$) and a higher frequency of α-thalassemia ($p < 0.05$), confirming previous studies[3-5]. Although the OST+ group exhibited higher Hb and Hct, and lower hemolytic component than the OST- group ($p < 0.01$), blood viscosity was not significantly different between the two groups ($p < 0.20$). In contrast, red blood cell (RBC) deformability ($p < 0.001$) and aggregation ($p < 0.05$) were increased in the OST+ group. The hydroxyurea (HU) treatment frequency was not significantly different between the two groups ($p < 0.20$). As HU is known to modulate RBC deformability[6], we analyzed the data as a function of HU therapy independently of osteonecrosis, and found that HU-treated patients had lower blood viscosity and greater RBC deformability (data not shown). Excluding HU-treated patients from the cohort did not change the results (table).

A binary (OST-/OST+) multivariate logistic model was used to identify factors associated with osteonecrosis in SCA patients, and included age, Hb, RBC aggregation and deformability, hemolytic component, α-thalassemia status and previous history of VOC as covariates. The overall model was significant (chi-square=30.192; df=7; $p<0.0001$), and retained age (OR: 1.06; 95% CI 1.01-1.12; $p<0.05$), Hb (OR: 2.24; 95% CI 1.19-4.18; $p<0.05$) and RBC deformability (OR: 1.15; CI 1.01-1.33; $p<0.05$) as independent factors statistically associated with osteonecrosis. Two other binary multivariate logistic models were

tested; one included the previous parameters plus blood viscosity and HU therapy, and the other excluded all HU patients. The results were similar to those in the first model (data not shown).

Our study demonstrates that increased RBC deformability is associated with osteonecrosis in SCA. Irregularly shaped, deformable sickle RBC were previously shown to be more adherent than rigid, irreversibly sickle RBC,[7] hence triggering vascular occlusion.[8] The greater RBC deformability found in the OST+ group is probably due to the greater frequency of α-thalassemia patients in this group since patients with α-thalassemia had greater RBC deformability (0.18±0.05) than patients without (0.15±0.06, $p<0.05$). Even though higher Hb levels were observed in patients with osteonecrosis, the data do not support a significant role for blood viscosity in the pathogenesis of this complication, even after excluding data from patients under HU therapy. Further studies will be required to delineate the mechanisms by which RBC deformability raise the risk for osteonecrosis.

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Authorship

Contributions: N.L., Y.L., M.R., M.D.H.D., D.M., X.W., B.T., M.L.L.M., M.E.J. and P.C. designed the research; N.L., Y.L., M.R., M.M.M., M.D.H.D., D.M., X.W. and P.C. performed the experiments; N.L., Y.L., M.R., V.T., B.T. and P.C. analyzed the results; N.L., Y.L., M.R., M.M.M. and P.C. interpreted the data; N.L., Y.L., M.R. and P.C. wrote the article; N.L., Y.L., M.R., M.M.M., M.D.H.D., V.T., D.M., X.W., B.T., M.L.L.M., M.E.J. and P.C. read and approved the final version of the manuscript.

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References

1. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. Blood Rev. 2007;21:37-47.
2. Mukisi-Mukaza M, Saint Martin C, Etienne-Julan M, Donkerwolcke M, Burny ME, Burny F. Risk factors and impact of orthopaedic monitoring on the outcome of avascular necrosis of the femoral head in adults with sickle cell disease: 215 patients case study with control group. Orthop Traumatol Surg Res. 2011;97:814-820.
3. Mukisi-Mukaza M, Elbaz A, Samuel-Leborgne Y, et al. Prevalence, clinical features, and risk factors of osteonecrosis of the femoral head among adults with sickle cell disease. Orthopedics. 2000;23:357-363.
4. Ballas SK, Talacki CA, Rao VM, Steiner RM. The prevalence of avascular necrosis in sickle cell anemia: correlation with alpha-thalassemia. Hemoglobin. 1989;13:649-655.
5. Milner PF, Kraus AP, Sebes JI, et al. Sickle cell disease as a cause of osteonecrosis of the femoral head. N Engl J Med. 1991;325:1476-1481.
6. Ballas SK, Dover GJ, Charache S. Effect of hydroxyurea on the rheological properties of sickle erythrocytes in vivo. Am J Hematol. 1989;32:104-111.
7. Mohandas N, Evans E. Adherence of sickle erythrocytes to vascular endothelial cells: requirement for both cell membrane changes and plasma factors. Blood. 1984;64:282-287.
8. Ballas SK, Larner J, Smith ED, Surrey S, Schwartz E, Rappaport EF. Rheologic predictors of the severity of the painful sickle cell crisis. Blood. 1988;72:1216-1223.
9. Nouraie M, Lee JS, Zhang Y, et al. The relationship between the severity of hemolysis, clinical manifestations and risk of death in 415 patients with sickle cell anemia in the US and Europe. Haematologica. 2012.
10. Baskurt OK, Boynard M, Cokelet GC, et al. New guidelines for hemorheological laboratory techniques. Clin Hemorheol Microcirc. 2009;42:75-97.

Table 1: General characteristics, hematological and hemorheological parameters

	With patients under HU		Without patients under HU	
	OST- (67)	OST+ (30)	OST- (57)	OST+ (22)
Age (yrs)	32.5 ± 12.2	39.3 ± 13.1*	32.0 ± 12.4	38.8 ± 11.9*
Sex (M/F)	32/35	11/19	27/30	9/13
HU (%)	15.9	27.6	-	-
α-thalassemia (%)	37.3	56.7*	40.4	59.1
Positive history of VOC (%)	9.0	26.7*	8.8	27.3*
HbF (%)	7.9 ± 5.7	9.6 ± 6.2	7.5 ± 5.6	9.1 ± 6.1
WBC (10^9/l)	9.5 ± 2.0	8.7 ± 2.1	10.0 ± 2.7	9.0 ± 1.7
RBC (10^{12}/l)	2.8 ± 0.6	2.9 ± 0.5	2.8 ± 0.6	3.1 ± 0.4
PLT (10^9/l)	404 ± 126	381 ± 136	414 ± 125	373 ± 144
MCV (fl)	83.5 ± 9.8	86.6 ± 10.1	81.4 ± 8.2	83.5 ± 7.4
MCHC (g/dl)	35.9 ± 1.1	35.6 ± 1.2	35.8 ± 1.1	35.5 ± 1.3
Hb (g/dl)	8.2 ± 1.3	9.0 ±1.1**	8.1 ± 1.2	9.1 ± 1.1***
Hct (%)	22.9 ± 3.7	25.2 ± 3.0**	22.7 ± 3.4	25.6 ± 3.1***
RET (%)	8.5 ± 3.3	7.7 ± 2.7	8.6 ± 3.3	7.7 ± 2.3
BIL (μmol/l)	61.9 ± 44.1	52.6 ± 37.4	62.8 ± 46.1	54.7 ± 43.0
AST (IU/l)	39.4 ± 14.8	37.0 ± 10.1	39.8 ± 14.3	37.1 ± 11.2
LDH (IU/l)	522 ± 166	433 ± 96**	537 ± 161	442 ± 100**
Hemolytic component (relative unit)	0.16 ± 1.10	-0.35 ± 0.61**	0.23 ± 1.08	-0.33 ± 0.61**
ηb ($mPa.s^{-1}$)	7.64 ± 1.79	8.24 ± 2.01	7.80 ± 1.75	8.40 ± 2.16
RBC deformability at	15 ± 6	20 ± 5***	15 ± 5	19 ± 5***

3 Pa (a.u.*100)				
RBC aggregation (%)	52 ± 9	57 ± 8*	52 ± 10	55 ± 7
RBC disaggregation threshold (s^{-1})	306 ± 148	262 ± 108	309 ± 152	265 ± 116

Means ± SD. All patients were at steady state at the time of the study, i.e., no blood transfusions in the previous three months and absence of acute episodes at least two months before inclusion into the study. Measurements of four hemolytic markers (bilirubin, BIL; lactate dehydrogenase, LDH; aspartate aminotransferase, AST; reticulocytes, RET) were performed using standard methods, and a principal component analysis was used to derive a hemolytic component value from these markers.[9] This standard statistical data reduction approach uses conventional clinical measurements to explain the maximum-shared variance among these indirect measures of hemolysis. The hemolytic component has recently been demonstrated to reflect intravascular hemolysis[9] and had a mean of 0 (SD = 1.0), and predicted 49.2% of the variation among all four measured variables (Eigenvalue = 1.97). Blood viscosity, red blood cell (RBC) deformability and aggregation properties were determined as previously described.[10] Polymerase Chain Reaction (Gap-PCR) was used to detect the 6 common α-thalassemia deletions, including $-\alpha^{3.7}$ and $-\alpha^{4.2}$ alleles, and triplication defects of the α-globin genes. HU, hydroxyurea therapy; VOC, vaso-occlusive crisis; HbF, fetal hemoglobin; WBC, white blood cells; RBC, red blood cells; PLT, platelets; MCV, mean cell volume; MCHC, mean corpuscular hemoglobin concentration; Hb, hemoglobin; Hct, hematocrit; ηb, blood viscosity; RBC, red blood cell. Significant difference between the two groups: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.